REMARKS

Reconsideration and allowance are respectfully requested in view of the Decmeber 31, 2002 Amendment and this Supplemental Amendment. The Examiner will appreciate that the December 31, 2002 Amendment included a petition to extend time.

Applicants attach abstracts of six (6) literature references that may pertain to the cytokine receptor super family

Amended claim 108 reflects editorial revision consistent with the original specification, and attention is respectfully directed to page 7, starting at about line 25. Chelating is further defined in new claim 136, which is also consistent with the original specification disclosure.

Amended claims 109, 112, and 113 reflect minor editorial revision consistent with the former claims and the specification as a whole. For instance, as to amended claim 109, attention is respectfully directed to exemplary description, such as at page 6 in the second paragraph, page 16 (table at pH 5), page 19 in the fourth paragraph.

Amended claim 119 finds basis in Figure 2. As graphically depicted, 0.1 ng of the substance, modified IL-3, may inhibit up to approximately 50% of 3ng/ml native IL-3. Amending claim 119 consistent with the Figure is therefore deemed compliant with 35 U.S.C.§112(¶1).

Amended claim 121 addresses informalities in the claim language.

Additional dependent claims 133, 134, 135 and 136 are based on subject matter previously in dependent claims 104, 108, 113 and 121. The new claims are therefore considered supported by the specification throughout, and as to new claim 135 the Examiner may also consider page 7, line 4 from the bottom to page 8, line 8.

Claims 125-128 have been canceled without prejudice to their presentation in a continuing application.

Applicants respectfully request the Examiner to enter these claim amendments, reconsider all objections and rejections, and pass this case on to allowance.

Respectfully submitted,

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Enclosure: Abstracts of literature references regarding cytokine super family.

CLAIMS AS AMENDED

Please amend and/or add claims:

108. (Amended) The method according to claim 94 or 99, wherein the modification **further comprises** ~~is performed by~~ reversibly denaturing the substrate and adding chelating agent to remove the metal ion~~, said chelating being conducted in the presence of urea and EDTA~~.

109. (Amended) The method according to claim 94 or 99, wherein the modification is specific for one type of amino acid**, specific to one amino acid,** or is specific for only one amine-residue in the peptide or protein

112. (Amended) A modified signal substance selected from the group consisting of a protein hormone, peptide hormone, growth factor, a haemopoeitic growth factor, an inteferon, an interleukin and a colony stimulating factor with enhanced biological activity, antagonistic activity or cell inhibitory activity, wherein said signal substance contains a modification within or in such close proximity to a catalytic center that it effects a biological or ~~bio~~chemical feature.

113. (Amended) A modified signal substance being a Zinc binding signal peptide selected from Growth Hormone, prolactin**,** insulin, and **a cytokine acting on a receptor** a ~~member~~ of the same ~~(~~cytokine~~)~~ **receptor** superfamily as the IL-3 receptor, said modified substance having **been modified in such close proximity to a Zinc binding center that the modified substance has acquired** an enhanced biological activity, antagonistic activity or cell inhibitory activity~~, wherein the modification is within or in such close proximity to a Zinc binding center that the metal binding properties have been changed~~.

119. (Amended) The substance according to claim 118, comprising at least one of the following characteristics

a) 0.1 ng of the substance, modified IL-3 inhibits up to **approximately** ~~about~~ 50% of 3ng/ml native IL-3;

b) 3 ng/ml of the substance, modified IL-3 suppresses 80-90% thymidine incorporation of 30-100 ng/ml control IL-3;

c) the substance modified IL-3 inhibits control IL-3 by a factor of 10-100.

121. (Amended) The substance according to one of claims 112-114, wherein the substance has acquired one of the following combinations of characteristics:

a) a decreased stability and increased antagonistic activity ~~for example acetylated IL-3~~;

b) a decreased stability and increased agonistic activity ~~e.g. N-terminally proteased IL-3 e.g. cathepsin C treated IL-3~~;

c) an increased stability and antagonistic activity ~~e.g. succinylated IL-3~~; **or**

d) an increased stability in combination with an agonistic activity ~~for example C-terminally proteased IL-3 e.g. Carboxypeptidase-Y treated IL-3~~.

Please add new claims as follows:

133. (New) The method according to claim 94 or 99, wherein said chemical modification comprises acetylation using Iodo acetate or succinylation using succinic anhydride, said chemical modification being conducted while gradually varying at least one of the conditions under which said chemical modification is conducted, said conditions comprising a pH range between a pH of 5.0 and 7.0, time for conducting said modification, and reagent concentrations.

134. (New) A modified signal substance according to claim 113, wherein said cytokine acting on the same cytokine receptor superfamily as the IL-3 receptor is selected from the group consisting of IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, GM-CSF, EPO, and IFN-gamma.

135. (New) The substance according to claim 121, wherein the substance acquiring one of said combinations of characteristics is

a) acetylated IL-3;

b) an N-terminally proteased IL-3;

c) succinylated IL-3; or

d) a C-terminally proteased IL-3.

136. (New) The method according to claim 108, wherein the chelating is conducted in the presence of urea and EDTA.

1: Baillieres Clin Haematol 1994 Mar;7(1):17-48

Cytokine receptors and signal transduction.

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The past few years have seen an explosion in the identification, cloning and characterization of cytokines and their receptors. The pleiotropic effects of many of the growth factors and the considerable redundancy in the actions of growth factors have contributed to a mass of descriptive literature that often seems to defy summary. Only recently have common concepts begun to emerge. First, cytokines mediate their effects through a large family of receptors that have evolved from a common progenitor and retain structural and functional similarities. Within the haematopoietic system, the cytokines are not usually instructive in differentiation, but rather supportive, and may contribute to some differentiation-specific responses. The patterns of expression of cytokine receptors are therefore a product of differentiation and provide for changes in physiological regulation. The second important concept that is emerging is that the cytokines mediate their mitogenic effects through a common signal-transducing pathway involving tyrosine phosphorylation. Thus, although the cytokine receptor superfamily members do not have intrinsic protein tyrosine kinase activity, by coupling to activation of tyrosine phosphorylation they may affect cell growth by pathways that are common with the large family of growth factor receptors that contain intrinsic protein tyrosine kinase activity. The coupling of cytokine binding to tyrosine phosphorylation and mitogenesis requires a relatively small membrane-proximal domain of the receptors. This region has limited sequence similarity which may be required for the association of individual receptors with an appropriate kinase. Activation of kinase activity results from the dimerization or oligomerization of receptor homodimers or heterodimers. Again this requirement is similar to that seen with the growth factor receptors which have intrinsic protein tyrosine kinase activity. The protein tyrosine kinases that couple cytokine binding to tyrosine phosphorylation are members of the Jak family of kinases. The ubiquitous expression of these kinases provides a common cellular background on which the cytokine receptors can function and on which unique functionally distinct receptors have evolved. In particular, tyk2 is required for the responses initiated by IFN-alpha while Jak2 has been implicated in the responses to G-CSF, IL-3, EPO, growth hormone, prolactin and IFN-gamma.(ABSTRACT TRUNCATED AT 400 WORDS)

Publication Types:
Review

Review, Academic

PMID: 7518712 [PubMed - indexed for MEDLINE]

2: EMBO J 1992 Dec;11(13):4909-15

Distinct downstream signaling mechanism between erythropoietin receptor and interleukin-2 receptor.

Yamamura Y, Kageyama Y, Matuzaki T, Noda M, Ikawa Y.

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Erythropoietin receptor (EPOR) and interleukin-2 receptor beta chain (IL-2R beta) belong to the same cytokine receptor superfamily and have highly conserved sequences in their intracellular signaling domain. However, common downstream signaling pathways of these receptors have not been demonstrated. In the present study, we introduced and expressed the murine EPOR in murine IL-2-, IL-3- and IL-5-dependent cell lines and analyzed their growth response to EPO. We found that the expression of EPOR induced EPO dependence in IL-3-dependent BAF-B03 and IL-5-dependent Y16 cells but not in IL-2-dependent CTLL-2 cells, although the EPOR-expressing CTLL-2 cell lines could bind and internalize EPO as efficiently as the BAF-B03-derived cell lines. Additional expression of AIC2B, a common signal transducer for IL-3R, IL-5R and GM-CSFR, made no difference to the EPO responsiveness of the EPOR-expressing CTLL-2 cell lines. These results suggest that the cellular components required for the transduction of EPOR signal and IL-2R signal are at least partially different, and this difference cannot be explained solely by the absence of AIC2B.

PMID: 1464316 [PubMed - indexed for MEDLINE]

3: J Biol Chem 1992 Jun 5;267(16):11619-25

Mutations in the Trp-Ser-X-Trp-Ser motif of the erythropoietin receptor abolish processing, ligand binding, and activation of the receptor.

Yoshimura A, Zimmers T, Neumann D, Longmore G, Yoshimura Y, Lodish HF.

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The erythropoietin receptor (EPOR) is a member of the newly identified cytokine receptor superfamily. A common sequence motif, Trp-Ser-X-Trp-Ser (WSXWS), near the transmembrane domain is highly conserved in this family. To determine the function of this motif, we constructed deletion and insertion mutations in this part of the EPOR and introduced them into an interleukin-3 (IL-3)-dependent hematopoietic Ba/F3 cell line. Cells expressing the wild-type EPOR displayed 1,500 erythropoietin (EPO)-binding sites/cell with a single affinity of about 300 pM and proliferate in the presence of IL-3 or EPO. Ba/F3 cells expressing receptors mutated in the WSXWS motif displayed little EPO binding on the cell surface and did not grow in the presence of EPO. The mutant receptors were retained in the endoplasmic reticulum (ER) and, as such, were unable to bind EPO. A single Gly insertion between the two WS sequences caused defects in receptor structure and function similar to mutations lacking all or part of the WSXWS motif. The EPOR can be activated, resulting in proliferation independent of EPO either by an Arg129 to Cys point mutation or by association with the Friend spleen focus-forming virus (SFFV) envelope glycoprotein gp55. Introduction of the point mutation (Arg129 to Cys) did not activate any of the receptors mutated in the WSXWS motif. Moreover, gp55 did not activate the mutant receptors in Ba/F3 cells. Our study indicates that the WSXWS motif is critical for protein folding, ligand-binding, and signal transduction.

PMID: 1317872 [PubMed - indexed for MEDLINE]

4: Proc Natl Acad Sci U S A 1992 May 15;89(10):4295-9

Cloning of the low-affinity murine granulocyte-macrophage colony-stimulating factor receptor and reconstitution of a high-affinity receptor complex.

Park LS, Martin U, Sorensen R, Luhr S, Morrissey PJ, Cosman D, Larsen A.

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A cDNA clone (clone 71) that encodes a low-affinity receptor for murine granulocyte-macrophage colony-stimulating factor (GM-CSF) has been isolated by direct expression. This molecule is the homologue of the human GM-CSF receptor alpha subunit, although homology between these molecules is surprisingly low (less than 35% amino acid identity). The cDNA encodes a polypeptide of 387 amino acids, which contains the conserved features of the hematopoietin receptor superfamily. When expressed in COS-7 cells, this clone encodes a protein that binds radiolabeled murine GM-CSF with low affinity. Coexpression of clone 71 with a cDNA corresponding to a low-affinity interleukin 3 (IL-3) receptor (AIC2A) did not alter the affinity of binding of either GM-CSF or IL-3. However, coexpression of clone 71 with the IL-3 receptor-related cDNA AIC2B generated

high-affinity binding sites for murine GM-CSF but not murine IL-3. These studies show that clone 71 and AIC2B are capable of forming an alpha beta complex capable of binding murine GM-CSF with high affinity, while AIC2A appears not to be a component of the murine GM-CSF receptor.

PMID: 1533931 [PubMed - indexed for MEDLINE]

5: Blood Rev 1991 Sep;5(3):193-203

The cytokine receptor superfamily.

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The binding of haemopoietic growth factors and cytokines to specific receptors triggers a cascade of intracellular events which results in cell proliferation and differentiation. The knowledge of ligand-receptor-signal pathways is not only important in understanding the pathophysiology of malignant disease but also essential for devising future therapeutic strategies. The advent of recombinant technology has made it possible to test the efficacy of selective differentiation therapy, and haemopoietic growth factors are undergoing clinical trials for a number of indications. In addition, increasingly the receptors for haemopoietic growth factors and cytokines have come under scientific scrutiny. Recently receptors for IL-2 alpha, IL-2 beta, IL-3, IL-4, IL-5, IL-6, IL-7, erythropoietin, G-CSF and GM-CSF have been isolated and cloned. It has become apparent that they have structural homology that is shared by receptors for growth hormone and prolactin, and this receptor group makes up the new cytokine receptor superfamily. The finding of sequence homology within these receptors suggests their evolutionary relationship. These receptors are transmembrane proteins 257-856 amino acids and their extracellular ligand-binding domain contains four conserved cysteine residues and a Trp-Ser-X-Trp-Ser motif. The secondary structure of the extracellular domain is made up of alpha-helices. High and low affinity binding forms exist for all these receptors. Binding affinity may depend on the formation of receptor heterodimers or multimers, association with other membrane proteins or differential glycosylation. Soluble receptor forms have been described for IL-2 alpha, IL-4, IL-5, IL-6 and IL-7. It is not known whether they are actively secreted or represent the degradation products of cell turnover. Their function may be to mop up excess cytokines and thereby confine the cytokine response. There is no sequence homology of the intracytoplasmic domains although several are rich in proline and serine residues, which may be important in mechanisms of signal transduction. No

receptor in this superfamily functions as a receptor tyrosine kinase or has intrinsic protein tyrosine kinase activity. Detailed study of individual receptors holds clues to the regulation of receptor expression, ligand-receptor interactions and mechanisms involved in signal transduction. Such knowledge might explain the pleotropic effects cytokines may have on different cell types and their overlap in biological functions. Elevated levels of soluble IL-2 alpha receptor (Tac) are detected in hairy cell leukaemia, lymphomas and adult T-cell leukaemia (TL), and levels reflect tumour burden.(ABSTRACT TRUNCATED AT 400 WORDS)

Publication Types:
Review
Review, Tutorial

PMID: 1663810 [PubMed - indexed for MEDLINE]

6: Ann Ist Super Sanita 1990;26(3-4):453-67

Receptors for interleukin 4, interleukin 5 and interleukin 6.

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cDNA encoding IL-4, IL-5 and IL-6 have been isolated and the availability of recombinant proteins allowed the demonstration of the pleiotropic effects of these cytokines. IL-4, IL-5 and IL-6 bind to high affinity receptors (Kd = 1-6 x 10(-10) M) expressed in low numbers on cells. The IL-4R is expressed on virtually every cell type whereas the IL-6R and IL-5R are less widely distributed. Low affinity IL-6 and IL-5 binding sites have also been detected but the biological activity of these different cytokines appears to correlate with the presence of high affinity binding sites. Biochemical characterization of the IL-4R, IL-5R and IL-6R by crosslinking of the labelled ligands, immunoprecipitation or purification experiments suggests that these receptors are composed of several proteins. IL-4 binds to three species (130, 75 and 65 kDa) and cDNAs encoding the 130 kDa protein have been isolated. IL-6 binds to a 80 kDa protein for which a cDNA has been isolated. Furthermore the IL-6 80 kDa protein complex binds to a 130 kDa transducer for which a cDNA was recently isolated. The IL-5R consists of three proteins of 105, 75 and 60 kDa but corresponding cDNAs have not yet been isolated. The IL-4, IL-6R 80 kDA protein and the IL-6 transducer protein display significant homology in their extracellular domains. These proteins belong to the new cytokine receptor family (hematopoietin receptors superfamily) which also includes the G-CSFR, GM-CSFR,

IL-3, IL-7R and IL-2R 70 kDa chain.

Publication Types:
Review
Review, Academic

PMID: 2091506 [PubMed - indexed for MEDLINE]